ANTIMICROBIAL AGENTS AND CHEMOTHERAPY, Dec. 1995, p. 2749–2751 Vol. 39, No. 12
0066-4804/95/$04.00+0

Open-Label Crossover Study To Determine Pharmacokinetics and Penetration of Two Dose Regimens of Levofloxacin into Inflammatory Fluid

J. CHILD,[1] D. MORTIBOY,[1] J. M. ANDREWS,[1] A. T. CHOW,[2] AND R. WISE[1]*

Department of Medical Microbiology, City Hospital NHS Trust, Birmingham, United Kingdom,[1] and The R. W. Johnson Pharmaceutical Research Institute, Raritan, New Jersey[2]

Received 27 March 1995/Returned for modification 30 July 1995/Accepted 15 September 1995

Two levofloxacin administration regimens were used for six healthy male volunteers. They received either 500 mg of levofloxacin orally every 12 h for five doses or 500 mg every 24 h for three doses, and then 6 weeks later they received the other course. The concentrations of the drug in plasma, cantharidin-induced inflammatory fluid, and urine were measured with a microbiological assay following administration of the final dose. Mean peak concentrations in plasma of 9.3 and 6.6 μg/ml were attained 1.1 and 1.2 h after the 12- and 24-h regimens, respectively. Mean peak concentrations in inflammatory fluid of 6.8 and 4.3 μg/ml were attained at 2.3 and 3.7 h, respectively. The average steady-state concentrations were 5.0 and 2.2 μg/ml in plasma and 4.7 and 2.3 μg/ml in inflammatory fluid, respectively. The mean terminal elimination half-lives for plasma were 7.9 and 8.0 h for the two regimens, respectively, and the same values were noted for inflammatory fluid. The overall penetration into inflammatory fluid ranged from 88 to 101% with the 12-h regimen and 83 to 112% with the 24-h regimen. Mean urinary recoveries were 87 and 86% over the corresponding interval of the 12- and 24-h regimens, respectively. These results suggest that administration of levofloxacin once and twice daily should be efficacious for infections caused by the majority of pathogens.

Levofloxacin is an oral quinolone, the levo isomer of ofloxacin. In vitro studies suggest that it is approximately twice as active as ofloxacin (3) and, compared with ciprofloxacin, has similar or enhanced activity against gram-positive organisms (3, 5, 9).

In this open-label crossover study, the pharmacokinetics and penetration of levofloxacin into an inflammatory exudate that mimics skin and soft tissue infections were examined following administration of 500-mg doses every 12 or 24 h. The penetration of this agent into an artificially induced inflammatory exudate was investigated by employing the cantharidin-impregnated plaster technique (14).

MATERIALS AND METHODS

Six healthy, Caucasian, male volunteers between 18 and 45 years of age (mean age, 28.5 years; mean height, 175 cm; mean weight, 76.6 kg) were selected. They had no history of serious illness; atopy, particularly drug allergy; or alcohol or drug abuse or an acute illness in the 7 days prior to the start of this study. They had not received any prescribed or over-the-counter medication in 3 days prior to the study or antibiotics in the 7 days prior to administration of the first dose.

Approval for this study was obtained from the Hospital Ethical Committee. After giving written informed consent, the volunteers gave a full history, underwent a full examination, and were shown to have normal routine hematological and biochemical profiles, normal urinalysis results, and a normal 12-lead electrocardiogram in the 2 weeks preceding the study period.

Each volunteer was randomly allocated to receive first either 500 mg every 24 h for three doses or 500 mg every 12 h for five doses.

On the first day of the trial, the volunteer's vital signs (blood pressure, pulse, respiratory rate, and oral temperature) were measured and routine hematological and biochemical profiles were determined again. Serum was obtained for a baseline levofloxacin assay.

The first dose was given under supervision with ~240 ml of water. The subjects were instructed to fast for 2 h before taking each dose and for 2 h after taking each dose according to the 12- or 24-h schedule.

On the evening of day 2, two 1-cm^2 cantharides-impregnated patches were applied to the forearm to induce blister formation. On the morning of day 3, an 18-gauge peripheral venous cannula (Viggo-Spectramed, Helsingborg, Sweden)

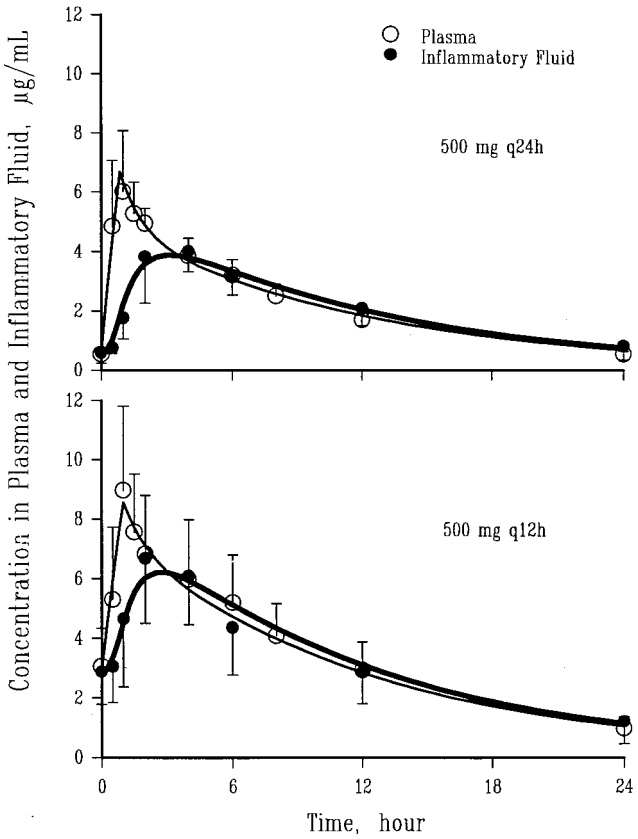

FIG. 1. Profiles of mean levofloxacin concentrations in plasma and inflammatory fluid following oral administration of 500 mg every 24 or 12 h for 3 days (last dose).

* Corresponding author.

TABLE 1. Levofloxacin pharmacokinetics[a] following oral administration of 500 mg every 24 or 12 h for 3 days (last dose)

Regimen	Plasma									
	C_{max} (μg/ml)	T_{max} (h)	T_0 (h)[b]	$t_{1/2}$ (h)[c]	$AUC_{0-\tau}$ (μg · h/ml)[d]	CL_d/F (liters/h)[e]	V_c/F (liters)[f]	V_{ss}/F (liters)[g]	C_{ss} (μg/ml)[h]	Goodness of fit (range)
500 mg every 24 h	6.55 ± 1.84	1.17 ± 0.52	1.14 ± 0.63	7.95 ± 1.35	53.5 ± 10.3	9.69 ± 2.08	66.4 ± 15.8	101.6 ± 13.0	2.23 ± 0.43	0.990 (0.98–0.99)
500 mg every 12 h	9.33 ± 2.27	1.08 ± 0.20	1.12 ± 0.35	7.91 ± 1.10	60.0 ± 18.2	8.92 ± 2.37	69.8 ± 18.6	97.5 ± 22.3	5.00 ± 1.51	0.96 (0.93–0.99)

[a] Mean ± standard deviation unless specified otherwise.
[b] T_0, duration of absorption.
[c] $t_{1/2}$, elimination half-life.
[d] $AUC_{0-\tau}$, AUC for administration interval.
[e] CL_d/F, apparent total-body clearance.
[f] V_c/F, apparent volume in central compartment.
[g] V_{ss}/F, apparent steady-state volume of distribution.
[h] C_{ss}, average steady-state drug concentration.
[i] V_T/F, apparent volume in peripheral compartment.
[j] A_u, total amount of drug collected in urine during administration interval.
[k] CL_r, renal clearance.

was inserted into the forearm without the blisters to facilitate the taking of serum samples throughout the course of the day; patency was maintained by flushing with 5 ml of 0.9% sterile saline (Antigen Pharmaceuticals, Roscrea, Ireland) after each blood sample was taken.

The final dose was then administered with ~240 ml of water. Seven milliliters of venous blood was collected immediately prior to and at 0.5, 1.0, 1.5, 2, 4, 6, 8, 12, and 24 h after administration of this dose.

Between 50 and 100 μl of blister fluid was aspirated with a fine needle prior to and 0.5, 1, 2, 4, 6, 12, and 24 h after administration of the final dose. The blister was then resealed with a plastic spray dressing (Smith and Nephew, Hull, United Kingdom). Urine was collected from 0 to 12 and 12 to 24 h after administration of the final dose.

After the final samples were obtained 24 h after dose administration, routine hematological and biochemical tests were repeated and the volunteers underwent a full medical examination.

Six weeks of "washout" and healing time was allowed to elapse before the second half of the crossover took place. Subjects were allocated to receive the opposite regimen from the previous trial period arm.

Drug analysis. Samples were kept in a light-proof cooler box at 4°C, transported to the laboratory, and analyzed within 1 h of being taken. Drug concentrations in plasma, blister fluid, and urine were measured by using a microbiological assay diffusion method. Assay plates containing IsoSensitest agar (CM471; Unipath, Basingstoke, United Kingdom) were flooded with a distilled water suspension of *Escherichia coli* 4004 (Bayer AG) prepared from an overnight broth culture and then diluted to an optical density at 630 nm of 0.004. Calibrators (2, 1, 0.5, 0.25, and 0.125 μg/ml), internal controls (1.5 and 0.2 μg/ml), and quality assurance samples were prepared in human plasma (Bradsure Biologicals, Market Harborough, United Kingdom), 70% human plasma in pH 7 phosphate buffer, and pH 7 phosphate buffer for assay of levofloxacin in plasma, blister fluid, and urine, respectively. Samples were applied to the plate in triplicate by filling 5-mm-diameter wells punched in the agar. After overnight incubation at 30°C, zones were measured with an image analyzer (Vidas; Image Associates, Thame, United Kingdom) and unknown concentrations were calculated from a calibration curve constructed by using Bennett's calculation (1). The lower limits of detection were 0.06 μg/ml in plasma and 70% human plasma and 0.03 μg/ml in pH 7 buffer. The between-assay coefficients of variation at concentrations of 1.5 and 0.2 μg/ml in plasma, 70% human plasma, and pH 7 buffer were 9.5 and 5.4%, 9.1 and 4.8%, and 9.9 and 6.3%, respectively. The within-assay coefficients of variation for plasma, 70% human serum, and pH 7 buffer were 8.9, 6.3, and 7.1%, respectively.

Pharmacokinetic analysis of the plasma and blister fluid samples was performed by the PCNONLIN program (10) with the Gauss-Newton algorithm and the Levenberg modification, as previously described (6). A two-compartment distribution model was fitted simultaneously to the plasma and blister fluid data of a subject. This model has been successfully applied to characterize the pharmacokinetics of levofloxacin following single- and multiple-dose oral administration (6). Plasma compartment absorption and elimination rates were assumed to be zero order and first order, respectively. The duration of absorption (T_0), area under the concentration-time curve (AUC) in plasma and inflammatory fluid per drug administration interval after administration of the final dose, apparent total-body clearance, apparent intercompartmental distribution clearance, apparent volumes in the central and peripheral compartments, apparent steady-state volume of distribution, average steady-state concentrations of levofloxacin in plasma and inflammatory fluid, and elimination half-lives in plasma and inflammatory fluid in the terminal phase were estimated by compartmental curve-fitting analysis. The maximum concentration (C_{max}) and the corresponding time (T_{max}) in plasma or blister fluid were determined by inspection. The degree of penetration into inflammatory fluid was estimated as the AUC ratios for the blister fluid and plasma per drug administration interval after the final dose was given. Steady-state urinary recovery was calculated as the total amount of levofloxacin in urine collected during a drug administration interval. Renal clearance was calculated by dividing the amount of levofloxacin recovered in urine during an administration interval by the AUC in plasma.

RESULTS

The mean concentrations found in plasma and inflammatory fluid following the once-daily and twice-daily regimens are shown in Fig. 1, and the derived pharmacokinetic parameters are in Table 1. The goodness of fit, that is, the correlation between the observed and predicted pharmacokinetic values, was ≥0.93 in all cases. This indicates that the model accounts for at least 93% of the variation and up to 7% can be attributed to error.

The concentration of levofloxacin in plasma prior to administration of the final dose was greater following twice-daily drug administration (3.1 μg/ml) than after once-daily administration (0.5 μg/ml). This is reflected in the 42% greater C_{max} in plasma in the group that received the drug more frequently. The T_{max} value were similar for both regimens, at approximately 1 h. The mean terminal (β) half-lives of elimination from plasma were similar at 8.0 and 7.9 h for the once-daily and twice-daily regimens, respectively, and the same values were noted for inflammatory fluid. The overall range was 6.0 to 9.8 h. The mean plasma AUCs at steady state for the groups receiving levofloxacin twice daily (60 μg · h/ml) or once daily (54 μg · h/ml) were very consistent.

Levofloxacin penetrated into the blister fluid rapidly; the T_{max} in this fluid generally occurred 1 to 2 h later than the T_{max} in plasma. The mean C_{max} values for blister fluid were 68 and 72% of those for plasma with the once- and twice-daily regimens, respectively. The average steady-state concentrations of levofloxacin in plasma and blister fluid, however, were very similar (2.2 and 2.3 μg/ml in plasma and blister fluid for the once-daily regimen and 5.0 and 4.7 μg/ml in plasma and blister fluid for the twice-daily regimen). The percentages of levofloxacin penetration into the exudate, calculated by comparison of the AUC per drug administration interval for measurements taken in inflammatory fluid with that for measurements taken in plasma, were similar at 100 and 93% for the once- and twice-daily regimens, respectively.

The total clearance of levofloxacin was apparently greater following the once-daily regimen (9.7 liters/h) than after the twice-daily regimen (8.9 liters/h), but the difference was not statistically significantly different (Student t test, $P > 0.1$).

TABLE 1—*Continued*

Inflammatory fluid									Urine	
C_{max} (μg/ml)	T_{max} (h)	$t_{1/2}$ (h)	$AUC_{0-\tau}$ (μg · h/ml)	CL_d/F (liters/h)	V_T/F (liters)	C_{ss} (μg/ml)	% Penetration	Goodness of fit (range)	A_u (% of dose)[i]	CL_r (liters/h)[i]
4.33 ± 0.96	3.67 ± 1.51	7.95 ± 1.35	54.1 ± 14.7	20.9 ± 10.1	35.2 ± 10.8	2.25 ± 0.61	100.2 ± 11.9	0.968 (0.953–0.994)	85.8 ± 8.1	8.28 ± 1.71
6.79 ± 2.05	2.33 ± 0.82	7.91 ± 1.10	55.9 ± 17.5	29.7 ± 11.8	27.7 ± 9.4	4.66 ± 1.46	93.0 ± 4.7	0.954 (0.867–0.996)	86.9 ± 21.8	7.70 ± 2.70

After administration of the final dose, a total of 429 mg of levofloxacin (86% of the dose) was recovered in the urine over the 24-h interval from those receiving the drug once daily and a total of 434 mg (87% of the dose) was recovered in the urine over the 12-h interval from those receiving 500 mg twice daily.

Physical examination revealed no abnormalities attributable to levofloxacin. Three volunteers reported a feeling of light headedness 40 to 60 min after receiving a dose of levofloxacin; this had a duration of about 1 h. The biochemical and hematological parameters studied revealed no abnormalities for the duration of the study.

DISCUSSION

There is little published information on the pharmacokinetics of levofloxacin at doses greater than 200 mg (2), but measurements of C_{max} and AUC suggest oral dose-response curve linearity from 50 to 200 mg. A study of the pharmacokinetics of both single and three times daily 350-mg doses of levofloxacin in patients with human immunodeficiency virus infection (6) showed a mean C_{max} of 6.92 μg/ml on day 7 following administration of 350 mg three times a day. Assuming dose-response curve linearity, this value is similar to our finding of a C_{max} of 9.3 μg/ml after administration of 500 mg twice a day. The elimination half-life we have reported (7.9 to 9.4 h) is slightly greater than that previously reported (6.3 h) (6), although the individual variations seen were somewhat greater in our study.

Fluoroquinolone bacterial killing rates are concentration dependent above the MIC (13), and a number of studies have been performed to ascertain the pharmacodynamic parameters which correlate with clinical efficacy (4, 7). The quotient of the AUC and the MIC is considered an appropriate parameter (12). Quotients of >125 for adequate efficacy and 250 for optimal efficacy have been suggested. Clinical trials will determine the appropriateness and efficacy of these various regimens.

The penetration of levofloxacin into blister fluid appears to be rapid and attains concentrations approximately two-thirds of those in plasma. The extent of penetration, about 100%, is similar to that of other fluoroquinolones, for example, sparfloxacin (117% [8]) and temafloxacin (104.5% [11]).

Our study suggests that levofloxacin, given as a once- or twice-daily dose of 500 mg, can attain concentrations in plasma and inflammatory fluid necessary to treat the majority of pathogens causing common systemic infections.

ACKNOWLEDGMENTS

We thank R. Hill of Cilag UK for advice and R. W. Johnson for financial support.

REFERENCES

1. **Bennett, J. V., J. L. Brodie, E. J. Benner, and W. M. M. Kirby.** 1966. Simplified, accurate method for antibiotic assay of clinical specimens. Appl. Microbiol. **14:**170–177.
2. **Davis, R., and H. M. Bryson.** 1994. Levofloxacin, a review of its antibacterial activity, pharmacokinetics and therapeutic efficacy. Drugs **47:**677–700.
3. **Dholakia, N., K. V. I. Rolston, D. H. Ho, B. LeBlanc, and G. P. Bodey.** 1994. Susceptibilities of bacterial isolates from patients with cancer to levofloxacin and other quinolones. Antimicrob. Agents Chemother. **38:**848–852.
4. **Drusano, G. L.** 1989. Pharmacokinetics of the quinolone antimicrobial agents, p. 71–105. *In* J. S. Wolfson and D. C. Hooper (ed.), Quinolone antimicrobial agents. American Society for Microbiology, Washington, D.C.
5. **Fu, K. P., S. C. Lafredo, B. Foleno, D. M. Isaacson, J. F. Barrett, A. J. Tobia, and M. E. Rosenthale.** 1992. In vitro and in vivo antibacterial activities of levofloxacin (*l*-ofloxacin), an optically active ofloxacin. Antimicrob. Agents Chemother. **36:**860–866.
6. **Goodwin, D. S., H. A. Gallis, A. T. Chow, F. A. Wong, S. C. Flor, and J. A. Bartlett.** 1994. Pharmacokinetics and safety of levofloxacin in patients with human immunodeficiency virus infection. Antimicrob. Agents Chemother. **38:**799–804.
7. **Hooper, D. C., and J. S. Wolfson.** 1991. Fluoroquinolone antimicrobial agents. N. Engl. J. Med. **324:**384–394.
8. **Johnson, J. H., M. A. Cooper, J. M. Andrews, and R. Wise.** 1992. Pharmacokinetics and inflammatory fluid penetration of sparfloxacin. Antimicrob. Agents Chemother. **36:**2444–2446.
9. **Marshall, S. A., and R. N. Jones.** 1993. The in vitro activity of DU-6859a, a new fluoropropyl quinolone. Antimicrob. Agents Chemother. **37:**2747–2753.
10. **Metzler, C. M., and D. L. Weiner.** 1992 PCNONLIN users' guide—version 4.1. Statistical Consultants, Lexington, Ky.
11. **Nye, K., Y. G. Shi, J. M. Andrews, J. P. Ashby, and R. Wise.** 1989. The in vitro activity, pharmacokinetics and tissue penetration of temafloxacin. J. Antimicrob. Chemother. **24:**415–424.
12. **Schentag, J. J., D. E. Nix, and A. Forrest.** 1993. Pharmacodynamics of the fluoroquinolones, p. 259–271. *In* D. C. Hooper and J. S. Wolfson (ed.), Quinolone antimicrobial agents, 2nd ed. American Society for Microbiology, Washington, D.C.
13. **Smith, J. T.** 1986. The mode of action of 4-quinolones and possible mechanisms of resistance. J. Antimicrob. Chemother. **18**(Suppl. 1)**:**21–29.
14. **Wise, R., A. P. Gillett, B. Cadge, S. R. Durham, and S. Baker.** 1980. The influence of protein binding upon tissue levels of β-lactam inhibitions. J. Infect. Dis. **142:**77–82.